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Yolk and copper utilisation during embryogenesis of the freshwater prawn *Caridina nilotica**

R PONNUCHAMY, S AYYAPPAN, S RAVICHANDRA REDDY and KATRE SHAKUNTALA
Department of Zoology, Bangalore University, Bangalore 560 001

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Abstract. Yolk and copper utilisation during embryogenesis of the fresh-water prawn *Caridina nilotica* var. *bengalensis* (De Man) have been described. The egg number/brood increased with increase in length as well as volume (L^3) of the mother animal. The water content increased from 61·3% (I stage) to 76·4% (III stage) as development proceeded. Fat appeared to be the major source of energy for embryonic metabolism. During development 28·4 μg of dry yolk substance were utilised for embryonic metabolism. The pattern of copper absorption followed the trend of salt absorption.

Keywords. Yolk; copper; embryogenesis; prawn; *Caridina nilotica*.

1. Introduction

The pattern of yolk utilisation during embryogenesis of organisms is known to reflect certain functional aspects of the embryo in relation to the environment (Needham 1931). Publications in this aspect of crustacean ecophysiology have indicated that the pattern of yolk utilisation varies considerably among different species (Pandian and Schumann 1967; Pandian 1967, 1970a, b; Katre and Pandian 1972; Vijayaraghavan and Easterson 1974; Vijayaraghavan *et al* 1976; Katre 1977a). Unlike in marine crustaceans, salts required for development of the embryo in freshwater either have to be made available in enough quantity along with yolk or have to be absorbed by the embryo against a concentration gradient (Needham 1950; Katre 1976). Being an important element of enzyme systems and respiratory pigments of crustaceans, the utilisation of copper during embryogenesis is also of considerable interest (Katre 1977 a). Work on the above aspects has so far been restricted to marine and estuarine crustaceans (Vijayaraghavan *et al* 1974) and more data on the freshwater crustaceans is needed (Katre 1977a). The present paper reports the pattern of yolk and copper utilisation during the embryogenesis of the freshwater prawn *Caridina nilotica*.

2. Materials and methods

Caridina nilotica var. *bengalensis* (De Man) occurs commonly in the freshwater habitats in and around Bangalore. For the present study, berried females were collected from Agram tank (near Bangalore) and all analyses were carried out in the laboratory. The total length of the females was measured from the tip of the rostrum to the tip of the telson. The egg mass was released from the plumose hairs of the pleopods on to a glass slide, the connections between the eggs was removed and the number of eggs/brood was counted. For chemical analyses, the following four stages of development were chosen :

Stage I. Soon after spawning. Eggs deep green in colour, oval in shape, oil globules prominent. Egg diameter at the widest region: $0{\cdot}509 \pm 0{\cdot}0764$ mm. Egg length at the longest axis: $0{\cdot}892 \pm 0{\cdot}0819$ mm (figure 1a).

Stage II. At one end of the egg a small portion is differentiated into the transparent blastoderm. Egg diameter: $0{\cdot}531 \pm 0{\cdot}0465$ mm; egg length: $0{\cdot}942 \pm 0{\cdot}0461$ mm (figure 1b).

Stage III. Pigmented eyes noticed. The egg more or less transluscent. Occasional beating of the heart observed. Egg diameter: $0{\cdot}609 \pm 0{\cdot}0427$ mm; egg length: $1{\cdot}005 \pm 0{\cdot}0495$ mm (figure 1c).

Stage IV. Freshly hatched zoea. Total length from tip of rostrum to tip of telson $2{\cdot}016 \pm 0{\cdot}1028$ mm (figure 1d).

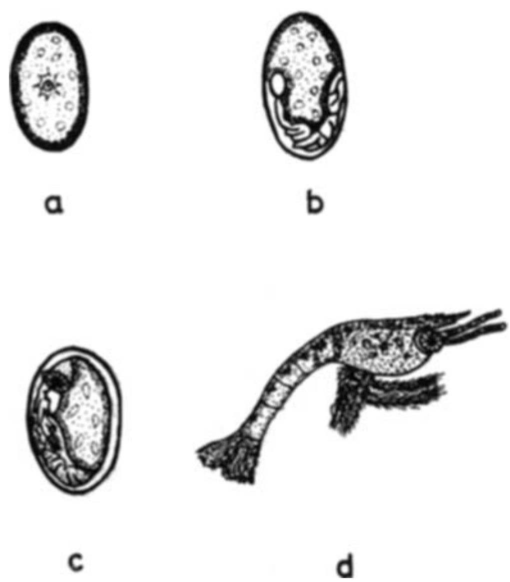

Figure 1. *Caridina nilotica* var. *bengalensis* : Different developmental stages ($\times$ 60) chosen for chemical analyses. (a) Egg soon after spawning. (b) Egg showing the differentiating blastoderm. (c) Egg just prior to hatching. (d) Zoea.

All analyses were made on material dried at 90° C to constant weight. Fat was estimated with Soxhlet apparatus according to the method detailed by the Association of Official Agricultural Chemists (1950); protein by the biuret method (Raymont *et al* 1964); ash by incinerating the sample in a muffle furnace at 560° C for 5 hr (Paine 1964) and energy by the method of Karzinkin and Tarkouskaya (1964). Copper was estimated in fresh tissue by the procedure detailed in Kolmer *et al* (1969).

3. Observations and results

Figure 2 indicates the relationship between the total length and weight of the gravid females. The relationship was linear conforming to the formula $Y = 111 \cdot 25 + 88 \cdot 55\ (X - 2 \cdot 35)$. The large deviations from the mean values, observed during the present studies are understandable because of variations in the egg number in relation to hatching time (Pandian and Katre 1972).

3.1. *Egg number in relation to mother animal*

A little harsh handling, jolting during transportation or storage of *Caridina* sp. may lead to the loss of eggs. Hence, care was taken to minimise handling of the prawns and avoid jolting during transportation. All egg counts were made on the day of collection. Considerable variation in the egg number even among the individuals of the same size group was encountered, which was mostly due to the hatching of some eggs of the brood carrying III stage eggs. Hence, for purposes of

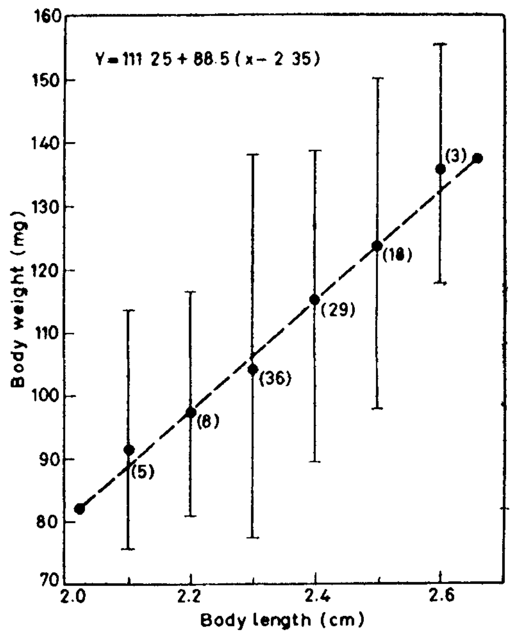

Figure 2. *Caridina nilotica* var. *bengalensis*: Length-weight relationship of the gravid females.

egg counts only broods with I or II stage eggs were counted. From table 1 it becomes clear that the egg number/brood increased with increase in length as well as volume (L^3) of the mother animal. Figure 3 indicates the regression analyses arrived at for the linear relationship between the volume of the mother animal and number of eggs carried in the brood. This agrees with the relationship suggested for marine malacostracans by Jensen (1958) and for freshwater prawns *Macrobrachium dayanum* (Koshy and Tiwari 1976), *M. rude* (Katre 1976) and *M. lamarrei* (Katre 1977b). It is apparent that for every increase in one unit of volume of *C. nilotica*, the egg number increases by 4·73. For *M. lamarrei* it was reported that for every 10 units of increase in volume, the egg number increased by 4·86 (Katre 1977b). The present increase in relation to single unit of volume is obvious due to the smaller total size attained by *C. nilotica* as compared to that attained by *M. lamarrei*.

Table 1. Egg counts from gravid females of *Caridina nilotica* of different body lengths (L) or volumes (L^3).

Number of females	L (cm)	L^3	Average number of eggs/broad
5	2·1	9·261	37·40 = 37
8	2·2	10·648	39·38 = 39
36	2·3	12·167	48·03 = 48
29	2·4	13·824	57·28 = 57
18	2·5	15·625	60·33 = 60
3	2·6	17·576	77·68 = 78

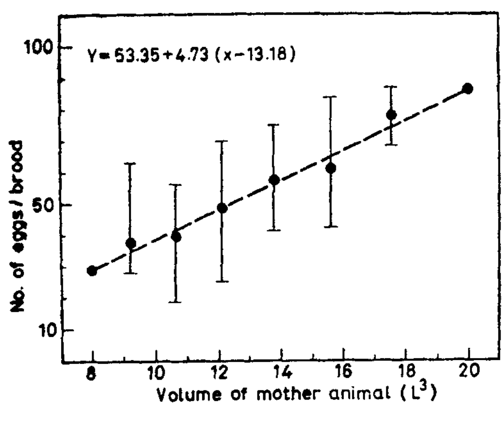

Figure 3. *Caridina nilotica* var. *bengalensis* : Relationship between the volume (length³) of the gravid female and number of eggs held in the brood.

3.2. *Changes in chemical composition of the eggs in relation to development*

Table 2 represents the percentage values of water, fat, ash, protein and caloric content in the different developmental stages of *C. nilotica*. With the advance in development from stage I to stage III, the eggs show increase in water content from 61·4 to 76·4%. This explains the increase in diameter from 0·509 to 0·609 mm. Such an increase appears to be a general feature of the developing eggs of crustaceans (Pandian 1970a; Vijayaraghavan and Easterson 1974) and the increase in total water of the egg indicates an osmotic hatching mechanism in this prawn also (Katre and Pandian 1972; Katre 1976; 1977a). During the corresponding period the ash (salt) content of the eggs also increased from 2·4 to 8·5% of the dry weight while the protein content of the egg did not show marked changes with respect to the advance in developmental stage. Fat content decreased from 69 to 62%. Corresponding to this there was a decrease in the caloric content per unit dry weight of egg/zoea. This indicates that fat was being utilised as a major source of energy during embryonic metabolism of *C. nilotica*. A similar trend towards fat utilisation also exists during the embryogenesis of another freshwater prawn *Macrobrachium lamarrei* (Katre 1977a).

3.3. *Changes in live and dry weights of eggs and zoea*

The live weight of a single egg of *C. nilotica* steadily increased from 210 μg in stage I to 281 μg in stage III (table 3). In the corresponding stages, however, there was a steady decrease in the dry weight from 78 to 66 μg/egg. On the whole, during development 28·4 μg of dry yolk substance was used for embryonic metabolism.

Table 2. Changes in chemical composition and caloric content of developing eggs of *Caridina nilotica*. Water content is given as a percentage of live weight; values of fat, ash and protein are given as percentages of dry weight and energy in cal/g dry substance (Values indicated in parentheses represent the number of estimates; ±SD)

Stage of development	Water	Fat	Ash	Protein	Energy
I	61·3 ± 5·33 (19)	68·8 ± 2·54 (6)	2·4 ± 0·04 (6)	28·8 ± 1·56 (6)	1496·6 ± 315·7 (6)
II	67·3 ± 2·91 (44)	60·5 ± 1·57 (6)	4·4 ± 0·12 (6)	34·9 ± 3·87 (6)	1418·9 ± 88·7 (6)
III	76·4 ± 3·81 (38)	60·0 ± 2·27 (6)	4·8 ± 0·04 (6)	35·2 ± 2·53 (6)	679·9 ± 111·4 (6)
IV	74·1 ± 1·83 (16)	61·6 ± 2·46 (6)	8·5 ± 0·11 (6)	29·9 ± 1·02 (6)	587·5 ± 32·9 (6)

Table 3. Live and dry weight of a single egg/zoea in the different developmental stages of *Caridina nilotica*.

Developmental stage	Number of prawns	Total number of eggs/zoea	Mean live weight (μg/egg or zoea)	Coefficient of variation %	Mean dry weight (μg/egg or zoea)	Coefficient of variation %
I	19	1168	200·7 ± 9·20	4·58	77·7 ± 4·00	5·14
II	44	2195	221·7 ± 12·00	5·41	72·6 ± 2·90	3·93
III	38	1979	281·0 ± 12·90	4·58	66·3 ± 4·00	6·03
IV	16	927	210·2 ± 3·27	1·53	49·3 ± 2·43	4·92

3.4. *Efficiency of yolk utilisation*

From the values presented in tables 2 and 3, average changes in chemical composition and caloric content of a single egg/zoea of *C. nilotica* are calculated and presented in table 4. Except for ash, all the other constituents of the eggs decreased as development progressed. Out of the total dry yolk expended on metabolic processes as much as 81·33% could be accounted for by oxidation of fat whereas only 18·67% through protein, again confirming the pattern of yolk utilisation reported earlier for *M. lamarrei* (Katre 1977a).

3.5. *Water, ash and copper metabolism*

A single egg of *C. nilotica* (200·7 μg live weight) requires as much as 80·3 μg of water for successful completion of embryonic development. Throughout the incubation period, the egg membrane appears to be permeable to water (see also Katre 1977a), unlike in marine crustaceans (Pandian 1970a b). During the corresponding period the egg also actively absorbed salts over a concentration gradient (table 5). Out of a total salt intake of 2·31 μg during the entire incubation period, as much as 0·37 μg was due to the absorption of copper. The pattern of copper absorption also followed the trend of salt absorption (figure 4).

4. Discussion

During the embryogenesis of *C. nilotica*, the efficiency with which the different substances of the yolk were utilised varied considerably. Such variations appear during the embryogenesis of all palaemonids (table 6). In the present study, fat was observed to be the main source of metabolic energy during the embryonic development of *C. nilotica*. The present observations differ from the concept proposed by Needham (1950) that during development, aquatic animals derive the energy from protein. The present observations also differ from those reported for *Macrobrachium idella* by Vijayaraghavan and Easterson (1974) and *Emerita*

Table 4. Chemical composition and caloric content of egg and zoea in the different developmental stages of *Caridina nilotica*.

Developmental stage	Water (μg)	Dry matter (μg)	Organic substance (μg)	Fat (μg)	Ash (μg)	Protein (μg)	Energy (cal)
I	123·00	77·70	75·84	53·47	1·86	22·37	0·3004
II	149·10	72·60	69·30	43·92	3·30	25·38	0·2847
III	214·70	66·30	63·12	39·80	3·18	23·32	0·1910
IV	160·90	49·30	45·13	30·38	4·17	14·75	0·1235

Table 5. Changes in ash and copper content of developing eggs and zoea of *Caridina nilotica*.

Developmental stage	Ash content		Copper content	
	μg/egg	% ash/egg	μg/egg	μg/g egg
I	1·86 ± 0·027	2·39 ± 0·035	0·152 ± 0·019	756·8 ± 98·5
II	3·30 ± 0·087	4·55 ± 0·120	0·227 ± 0·040	1025·6 ± 181·2
III	3·18 ± 0·027	4·79 ± 0·040	0·504 ± 0·051	1794·9 ± 181·2
IV	4·17 ± 0·053	8·45 ± 0·107	0·521 ± 0·073	2476·7 ± 314·1

holthusi (Vijayaraghavan *et al* 1976) where protein was shown to be the major energy source during development. It is of interest to note that even within the Palaemonidae there is a difference in the pattern of yolk utilisation during embryogenesis. The present observation of fat being the main energy source during the embryonic development of *C. nilotica* conforms with that reported for several other animals, viz., *Loligo vulgaris* (Stolfi 1933), *Crangon crangon* (Pandian 1967) and *Crepidula fornicata* (Pandian 1969).

Though marine crustacean eggs are initially impermeable to water, Pandian (1970a, b) reported that they were permeable to salts throughout their development. In the eggs of *C. nilotica* too, salts are absorbed throughout the incubation period. A similar pattern was also reported for *M. lamarrei* (Katre 1977a). Assuming the initial live weight of the egg to be 1 mg, the water requirement of marine eggs range from 70 μg in the isopod *Ligia oceanica* to 2129 μg in the lobster *Homarus americanums* (Pandian 1970b). The corresponding range for salts was 55 μg for the shrimp *Crangon crangon* to 67·5 μg for *L. oceanica*. Comparable values calculated for the eggs of *C. nilotica* are 406 μg of water and 11·1 μg of salt, and these

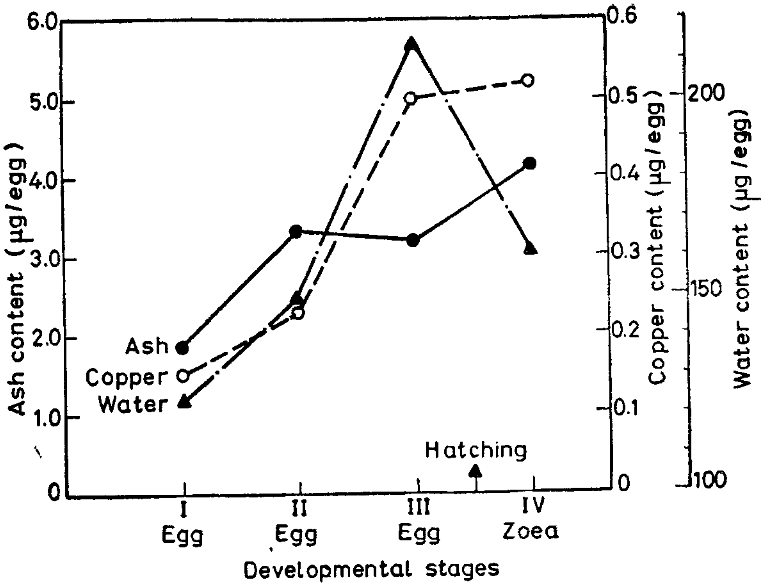

Figure 4. *Caridina nilotica* var. *bengalensis* : Changes in ash (= salt), copper and water contents of a single egg/zoea, in relation to development.

Table 6. Cumulative efficiencies of utilisation of various components of the yolk during embryogenesis of some *Palaemonidae*.

Constituents	Per cent efficiency in		
	Macrobrachium idella Recalculated from Vijayaraghavan and Easterson 1974	*Macrobrachium lamarrei* From Katre 1977a	*Caridina nilotica* Present work
Dry matter	61·5	72·9	63·5
Organic matter	..	69·9	59·8
Carbohydrate	80·7	..	..
Fat	26·6	51·7	56·8
Protein	47·6	91·9	61·5
Energy	43·6	65·9	41·1

lie in the above range and are also comparable to the values reported for *M. lamrrei* (382 μg and 10·4 μg respectively; Katre 1973). In *C. nilotica* the gross efficiency during development was 39·3% and the energy loss was 60·74% (table 7). According to Brody (1945), the gross efficiency for development in animals is known to remain more or less constant in spite of the differences in the size of animals belong-

Table 7. Percent gross efficiency (GE) and energy loss (EL) in some crustaceans during embryonic development.

Species	GE	EL	Reference
Eupagurus bernhardus	83·94	16·06	Pandian and Schumann 1967
Crangon crangon	82·05	17·95	Pandian 1967
Homarus gammarus	64·68	35·32	Pandian 1970a
Homarus americanus	85·62	14·38	Pandian 1970b
Ligia oceanica	97·96	2·04	Pandian 1972
Macrobrachium idella	44·04	56·00	Vijayaraghavan and Easterson 1974
Macrobrachium lamarrei	46·15	53·85	Katre 1977a
Emerita holthusi	95·27	4·23	Vijayaraghavan *et al* 1976
Caridina nilotica	39·26	60·23	Present work

ing to the different species. However, from table 7 which also indicates the percentage of gross efficiency and energy loss of other crustaceans it is interesting to note that the values differ widely and indicate the capacity with which the available yolk is utilised. Further it is also evident that the gross efficiency decreases in estuarine/freshwater species as compared to marine ones.

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